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RESEARCH PAPER

Conspecific alarm cues induce an alternative reproductive strategy in aquatic oligochaetes

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Abstract Although strategies that increase the survival of a predator attack have been widely observed, the strategy of altering reproductive effort under predation threats has not been well documented. A switch to sexual reproduction or an increased asexual reproductive rate may be inducible antipredator strategies in animals that are capable of both asexual and sexual reproduction. The results of laboratory experiments with two oligochaete species, *Stylaria lacustris* (L.) and *Nais christinae* Kasprzak, indicated that an increased asexual reproductive rate, a less typical strategy in response to alarm cues from conspecifics, is employed by the Naididae worms. The oligochaetes of both species exposed to conspecific cues also used the additional strategy of increased length at fission and consequently the increased length of parental worms and descendants after fission. The increase in body length appears to be beneficial in the case of sublethal predation by invertebrates. The similar life history changes observed in these two oligochaete species may be a universal adaptation against diverse predators that are abundant in the littoral zone.

Keywords Alarm cues · Asexual reproduction · Life history shifts · Naididae

Introduction

Many studies have established that defense mechanisms against predators evolved as adaptations to reduce the encounter probability or vulnerability to being consumed (Edmunds 1974; Havel 1987; Lima and Dill 1990). Most behavioral, morphological, physiological and life history defenses result in an increase in prey survival under predation pressure. Defense mechanisms are widely considered as costly (e.g., in terms of energy budget or time spent foraging, Skelly 1992; Baldwin 1996; DeWitt et al. 1998), but the absence of defense mechanisms may result in the death of the prey before they begin to reproduce. Most investigations of the fitness cost of antipredator adaptations, such as altered behavior or changes in life history, which reduce vulnerability to predators, demonstrated their negative effect on prey growth and reproduction (McCollum and Van Buskirk 1996). However, several studies show that prey can increase their reproductive effort and produce more offspring in risky habitats (Reznick and Endler 1982; Dawidowicz and Loose 1992; Macháček 1993). Prey species that increase their reproduction may benefit in the form of higher offspring numbers. A greater number of offspring increases the reproductive success and probability that at least some of the offspring survive. However, there are costs associated with increased reproduction: decreased growth rate, longevity and future fecundity (Warner 1984; Frankino and Juliano 1999; Forsman 2001). These costs can be reduced when potential prey employ antipredator strategies facultatively in the presence of predator.

Inducible defenses can be observed in animals that are able to assess the local level of predation. Many antipredator adaptations are induced by predator-released

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kairomones or by chemical cues from injured prey (Chivers and Smith 1998; Kats and Dill 1998). A large group of animals that are suggested to respond to kairomones or alarm cues are clonal invertebrates, such as cladocerans, several rotifers and aquatic oligochaetes (Gilbert 1980; Dodson and Havel 1988; Kaliszewicz and Uchmański 2009a, b). In contrast to parthenogenetic cladocerans and rotifers, oligochaetes belonging to the Naididae family are clonal animals that reproduce asexually through paratomy fission without the involvement of gametes. In such animals, an increase in the reproductive rate is related to an increase in the individual growth rate (Bak et al. 1981; Ryland et al. 1984; Han and Uye 2010). However, an increase in the growth rate during vegetative reproduction, such as fission or budding, does not directly increase the body size of the parental individual. Thus, a trade-off between the investment in asexual reproduction and body size exists.

The purpose of this study is to test the hypothesis that an increased asexual reproductive rate is an inducible antipredator strategy used by animals capable of both sexual and asexual reproduction and to discuss the potential costs and benefits of this strategy. Fission or budding appears to enable an animal to perform quick changes in its reproductive rate and, consequently, a rapid response to predator presence. Alternatively, the clonal animals that reproduce by vegetative means may prefer strategies that reduce the chance of being consumed, e.g., changes in body size. I conducted laboratory experiments to explore the life history changes in two aquatic oligochaete species, *Stylaria lacustris* and *Nais christinae* (Naididae, Oligochaeta), exposed to conspecific cues. Both species exhibit similar life history strategies, coexist and are widely distributed in lakes and ponds in the temperate zone. During spring, summer and early autumn they reproduce exclusively asexually through paratomy fission, which leads to many clonal generations (Brinkhurst and Jamieson 1971; Christensen 1984). Under unfavorable conditions, which are caused by low temperatures, they switch to sexual reproduction, deposit cocoons and then die. In spring, new generations of oligochaetes hatch from eggs and begin asexual fission. *S. lacustris* is a large species (18 mm) often dominating oligochaete assemblages in the littoral zone of eutrophic lakes, and it is subject to predation by damselfly larvae, Tanypodinae midge larvae, leeches, flatworms and hydras (Thompson 1978; Davies et al. 1981; Young 1981; Koperski 1998). *N. christinae* is a smaller species (5–8 mm) than *S. lacustris* and may be vulnerable to different predators or the young stages of the same predatory species.

Methods

Response of clonal oligochaetes to conspecific cues

Oligochaetes were reared in the laboratory for several clonal generations before use in the experiments. From 2002 to 2004, the initial worms were collected from the littoral zone of the eutrophic Lake Dziekanowskie (central Poland), where they coexist with fish and many invertebrate predators (predatory insect larvae, leeches, flatworms and hydras). To obtain the clones, the culture was initiated by placing single worms in individual containers in the laboratory after they had been collected in the field. The oligochaetes were maintained at a temperature of 19–20 °C for a maximum of 3 years. The increase in the number of individuals was monitored, and different clones were maintained. The samples were kept in the laboratory in 300-ml containers filled with water from the lake. The water was first sieved through a 30-μm mesh to exclude planktonic organisms and then underwent mechanical and active carbon filtration to remove microzoo- and microphytoplankton, odors and pollutants. The water was then held in tanks for 2 weeks to allow the degradation of chemical cues. The water with no alarm cues was used for the worm culture and for control treatments. The water was replaced every 4 days in the culture containers. Oligochaetes were fed with detritus originating from the same lake that had been collected at a depth of 0.5 m. Before use, the detritus was sieved through 1-mm mesh, settled in an aquarium, portioned and frozen. This procedure allowed the elimination of benthic invertebrates, which cannot be ingested with detritus by the oligochaetes. The oligochaetes used for the experiments were of similar age without visible fission zones; the *S. lacustris* worms were 9–11 mm in length, and the *N. christinae* were 4–5 mm in length.

Two experiments were performed on two selected clones that were well adapted to laboratory conditions. *S. lacustris* and *N. christinae* were exposed to conspecific alarm cues. *S. lacustris* individuals ($n = 20$ per treatment) were placed separately in 150-ml containers. Each container was filled with 50 ml of lake water and included 2 ml of detritus as the source of food. The chemical cues were generated by crushing 32 *S. lacustris* individuals (approximately 9 mm in length) in a small volume of water and then diluting it to obtain a final volume of 1 l. The water was withdrawn from the experimental containers each day and replaced with water containing chemical alarm cues or with water with no cues (control treatments). The control worms were not exposed to any chemical signals. *N. christinae* individuals ($n = 10$ per treatment) were placed separately in 25-ml containers. Each container

was filled with 15 ml of water and included 0.5 ml of detritus as the source of food. The procedures of generating the alarm signals from *N. christinae* were the same as those described for *S. lacustris* and other animals (Pijanowska 1997; Keppel and Scrosati 2004; Kaliszewicz and Uchmański 2009b). The chemical cues were generated by crushing 33 individuals (approximately 4 mm in length) per 1 l of water on each day of the experiment. The control worms were not exposed to chemical cues. The containers were maintained at a temperature of 20.3 ± 0.3 °C [mean ± 95 % confidence interval (CI)] under a 16L:8D photoperiod. The experiments lasted until a second generation of descendants appeared, which was 20 days for *S. lacustris* and 18 days for *N. christinae*. The parental worms were measured under a binocular microscope each day of the experiment to a precision of 0.5 mm. The presence of fission zones and the number of descendants were noted.

For each experiment, the following life history traits were measured: (1) the fission rate calculated as the number of clonal offspring per parental worm divided by the time; (2) the number of multiple fission zones whose presence was associated with the increased fission rate; (3) length of the parental worm at fission; (4) fission ratio calculated as the length of the parental worm after fission divided by its length before fission. A lower fission ratio indicates the size scale shift toward larger descendants after fission. A larger body size of the parental worm and descendant can increase their chance of survival in two ways: the larger animal outgrows some of its predators or has a greater chance to be injured; the lost body part from a predator attack can then be regenerated (Kaliszewicz 2003).

Statistical analyses

The data were analyzed using a multivariate analysis of variance (MANOVA) for four dependent variables: the fission rate, number of multiple fission zones, length of the parental worm at fission and fission ratio. The effect of the chemical cues on each life history trait was analyzed using a *t* test (two sets of data). A significance level of $\alpha = 0.05$ was used for the statistical analysis. All statistical analyses were performed using *Statistica* (Statsoft Inc).

Results

Life history changes induced by conspecific cues

The multivariate analysis showed significant effects of alarm cues (MANOVA, $F_{5,11} = 10.55$, $P < 0.001$) on life history traits in prey. The exposed *S. lacustris* worms increased their reproductive rate and length at fission compared with the control worms (*t* test, $t = 6.09$, $df = 18$, $P < 0.001$, $t = 3.03$, $df = 15$, $P < 0.01$, respectively; Fig. 1A, C).

Significantly more multiple fission zones in the *S. lacustris* oligochaetes were observed in the cue treatment than in the control treatment (*t* test, $t = 7.09$, $df = 18$, $P < 0.001$; Fig. 1B). The formation of several fission zones (two or three; Fig. 2) at the same time in one individual is a process that decreases the time between fission events and allows the worm to increase its reproductive rate. By contrast, there was no significant effect of alarm cues on the fission ratio (*t* test, $P > 0.05$; Fig. 1D). The fission ratio reflected the size of the descendants whose average sizes were 6.0 and 6.3 mm for the control and exposed oligochaetes, respectively.

The *N. christinae* worms also detected and responded to alarm cues from conspecifics (MANOVA, $F_{6,7} = 6.57$, $P = 0.012$). *N. christinae* exposed to alarm cues exhibited both a higher fission rate (*t* test, $t = 2.59$, $df = 15$, $P = 0.02$) and a longer body at fission than the control individuals (*t* test, $t = 2.23$, $df = 14$, $P = 0.04$; Fig. 1E, G). The number of multiple fission zones in the oligochaetes was higher in the cue treatment than in the control ($t = 2.15$, $df = 15$, $P = 0.04$; Fig. 1F). The increased number of fission zones affects the individual reproductive rate. In contrast to *S. lacustris,* the *N. christinae* worms exposed to alarm cues significantly changed their fission ratio in comparison to the control worms (*t* test, $t = 2.53$, $df = 15$, $P = 0.02$; Fig. 1H). The fission ratio indicated that the descendants were larger (3.9 mm) compared to the control worms (3.4 mm).

Discussion

The results of this study indicated that both oligochaete species, *S. lacustris* and *N. christinae*, altered their life history in response to alarm cues from conspecifics. These cues have been reported as a signal of the presence of an active predator for many aquatic invertebrates and fish (e.g., Wisenden et al. 2001; Mirza et al. 2003; Laforsch and Beccara 2006). The crucial life history shift observed in the oligochaetes of this study was an increase in the fission rate. The increased asexual reproductive rate can be regarded as an antipredator adaptation and as an alternative strategy to sexual reproduction. In risky habitats, animals that are capable of both modes of reproduction, e.g., freshwater zooplankton, particularly *Daphnia magna* (Ślusarczyk 1999; Walsh 2013), may benefit from switching from asexual to sexual reproduction. Sexual reproduction leads to the production of resting eggs and avoids the seasonal risk of predation. However, the Naididae oligochaetes employed the opposite strategy—they intensified

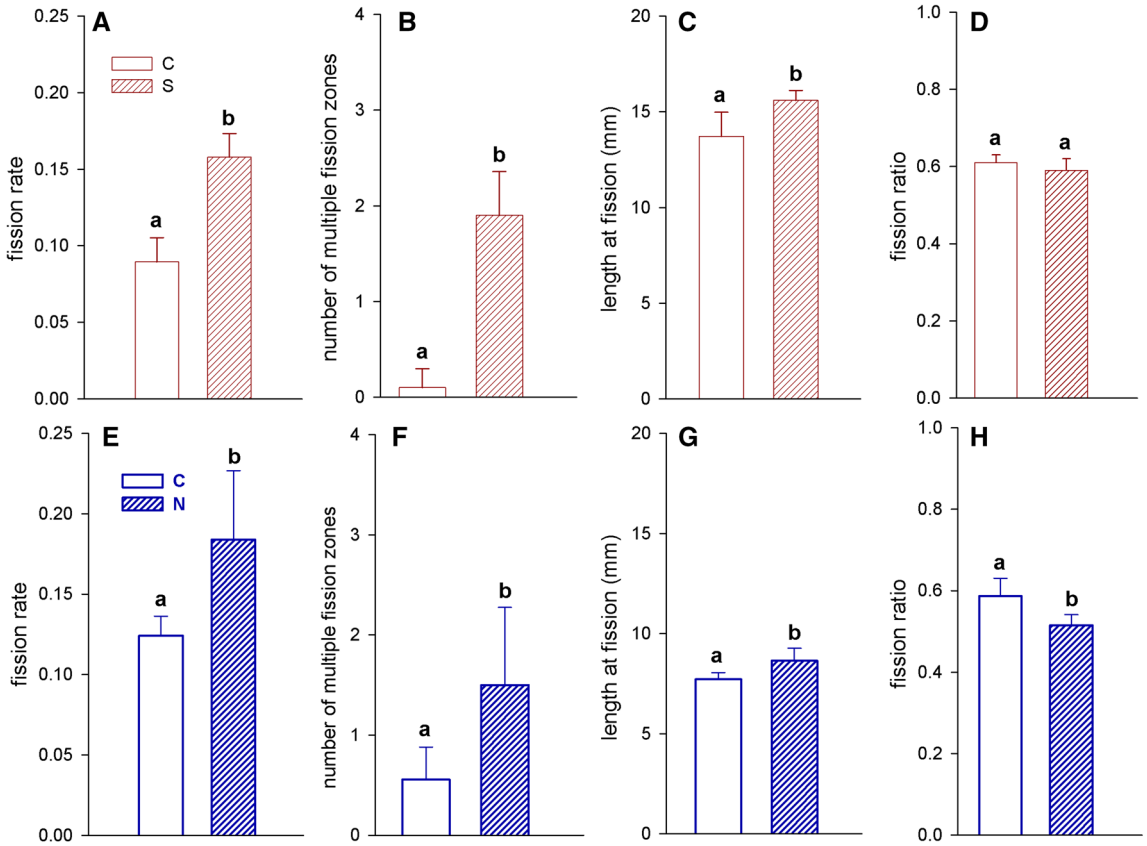

Fig. 1 Effect of alarm cues from crushed conspecifics on *Stylaria lacustris* (*S*) and *Nais christinae* (*N*) life history traits, such as the following: fission rate (calculated as the number of descendants divided by the time of reproduction; **A**, **E**), the number of multiple fission zones (**B**, **F**), length at fission (**C**, **G**) and fission ratio (**D**, **H**). *C* control worms. *Bars* represent 95 % confidence intervals (CIs). *Bars* sharing the *same letter* are not significantly different ($P > 0.05$)

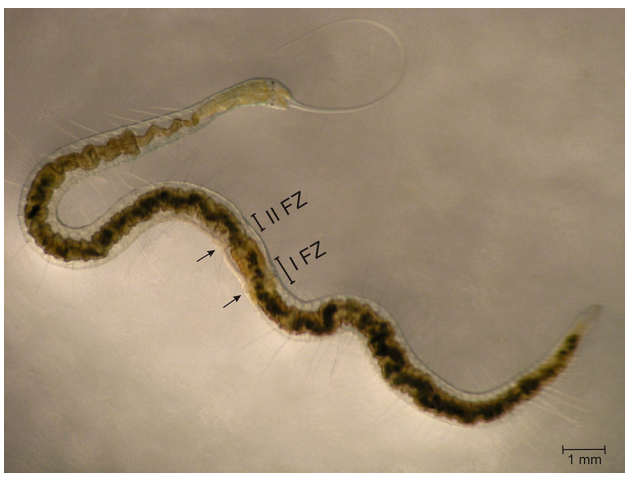

Fig. 2 The asexual *Stylaria lacustris* oligochaete with two fission zones. Fission zones appeared in the middle of the body where the new pygidium of the parental worm developed and the head region and trunk segments of the descendant developed. Fission furrows are indicated by the *arrows*. *IFZ* the first fission zone, more advanced in the development of the descendant; *IIFZ* the second fission zone

asexual fission. Sexual reproduction limits the number of offspring compared with the hypothetical number of asexual descendants that could be produced by an individual if they do not multiply sexually. This trade-off appears to be offset by the intensity of predation pressure and the probability of survival for adults and their young. Offspring that hatch from parthenogenetic or sexual eggs are significantly smaller than the adults and usually vulnerable to many predators. Offspring that are produced through paratomic fission are almost half as large as the parental worms. This increases their chance to survive in risky habitats. If predator pressure is low or medium, an optimal strategy appears to be the production of asexual offspring, which increases the reproductive success compared with sexual reproduction. Under high predation pressure, particularly seasonal, the production of resting eggs appears to be an optimal strategy. However, in the case when an adult animal can survive predator attacks, the high predation pressure does not seem to result in a switch from asexual to sexual reproduction. Oligochaetes that are highly capable of regeneration can survive the predator

attack and then regenerate the injury. Sublethal predation effects have been reported for many oligochaetes including *Stylaria lacustris* (Kajak and Wiśniewski 1966; Giere and Pfannkuche 1982; Kaliszewicz 2003). The increased asexual reproductive rate observed in the studied Naididae oligochaetes increases the chance that some offspring will survive but does not increase the survival probability of the parent itself. Thus, a question arises: why do prey use a strategy that does not increase their own survival under the threat of predation?

It is possible that the prey cannot employ a successful antipredator strategy to increase their survival in some environments, such as in habitats with many diverse predators. Different predators have different potential impacts on prey, and situations where prey face several predators simultaneously are expected to be quite common in nature. Previous studies have demonstrated that prey defend against the most dangerous predator in their habitat (Relyea 2003), but they can still be vulnerable to many less abundant predators. Moreover, if growing prey are similarly vulnerable to predation at different life stages, the changes in life history, e.g., size at maturity, cannot be applied successfully as an antipredator strategy. When the reduction of prey vulnerability to predators is limited, the beneficial adaptation appears to be a simple increase in the reproductive output. The increase in the offspring number increases the probability that at least some of the offspring survive regardless of the mode of predation. However, the strategy of increasing the reproductive rate does not increase the survival probability of parental worms. It is likely that they will die before they begin to reproduce in high-risk predation scenarios. This type of antipredator strategy, which does not increase the survival of the animal, requires future research into the context of increased reproduction and its consequences for potential prey.

In this study, oligochaetes exposed to conspecific alarm cues not only increased their asexual reproductive rate, but also increased their length at fission. These changes in life history may be similar to the increase in size at maturity in animals reproducing with the involvement of gametes. When selective predators prey upon small animals, delayed maturity and an increase in size at reproduction allow prey to escape with a large body size and to decrease their vulnerability to size-selective predators. This antipredator strategy has been reported for fish, snails and most planktonic cladocerans that were subject to invertebrate predation (Crowl and Covich 1990; Lüning 1992; Belk 1998). The increase in the length at fission implies that clonal oligochaetes can be under size-selective predation pressure. Previous studies have revealed that predation has a size-selective effect on *S. lacustris*; small worms were mainly eaten completely by predators, whereas larger ones were only damaged in most cases (Kaliszewicz 2003; Kaliszewicz et al. 2005). The increase in size at fission allows the worms to survive predator attacks and then regenerate the injury. The increase in length at fission increases the size of both the parental worm and the descendant after fission. Moreover, the changes in the fission ratio observed in *N. christinae*, a smaller species than *S. lacustris*, exposed to alarm cues indicated that these oligochaetes increase the size of the descendants under predation threat by increasing their length at fission and by manipulating the fission ratio.

The increase in asexual reproductive rate and body length can benefit prey under unpredictable predation threats and should be common in animals that coexist with diverse predators and that can easily manipulate the number of offspring. Oligochaetes are animals that appear to fit this description. Sublethal predation and regenerative capabilities increase their chance to survive in risky habitats. In the case of switching from asexual to sexual reproduction, oligochaetes may lose the anterior part of the body where the gonads are located in the predator attack. This can reduce the reproductive success more than if they reproduce asexually. The morphological and life history traits of Naididae oligochaetes appear to indicate that increased asexual reproduction is an optimal antipredator strategy.

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